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Studies of Composition and Crystallinity of Bacterial Poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate)

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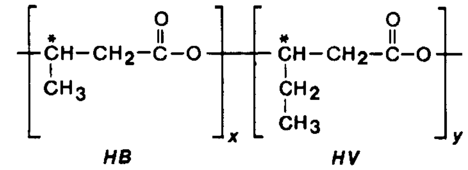
ABSTRACT: Samples of bacterial poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV)) were analyzed by 250-MHz ^{1}H nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopy. The compositions of 40 copolyester samples, as determined by NMR, ranged from 0 to 47 mol % β-hydroxyvalerate (HV). The shapes and intensities of numerous IR bands, particularly those at 1279, 1228, and 1185 cm^{-1}, were found to be sensitive to the degree of crystallinity. FTIR bands sensitive to composition include the C–H bands around 2900 cm^{-1} and the C–C band at 977 cm^{-1}. By use of the 2900-cm^{-1} bands, methods were developed for composition analysis of as-received samples of equal degree of crystallinity in the solid state and in solution. Relative areas, in which the C–H area was normalized to the composition-insensitive C═O area, were calibrated to the copolymer compositions determined by 250-MHz ^{1}H NMR. The experimental uncertainties of composition analysis of P(HB-*co*-HV) by NMR and FTIR were estimated to be ±1 and ±2–3 mol % HV, respectively. FTIR and wide-angle X-ray diffraction (WAXS) studies on solution-cast and melt-quenched samples showed a dramatic trend in that the rate of crystallization of these copolyesters decreases with increasing HV content. A two-stage crystallization process was identified for P(HB-*co*-HV) cast from solution and was attributed to changes in both nucleation rate and rate of crystal growth, whereas crystallization from the melt is largely nucleation rate limited. The crystallinity index (CI) determined by FTIR for as-received samples of P(HB-*co*-HV) was nearly independent of HV content, indicating that the copolyesters are as highly crystalline as PHB homopolymer. The degree of crystallinity (X_c) derived from X-ray diffraction was used to follow crystallization from the melt. The final value of X_c ranged from 62 to 69% for equilibrated copolyesters quenched from the melt. Crystallization of these copolyesters from the melt is faster than for solution-cast samples. However, both processes are slow when compared to the rate of crystallization of solution-precipitated samples.

Introduction

Poly(β-hydroxybutyrate) (PHB) is a naturally occurring polyester that constitutes a carbon reserve in a wide variety of bacteria.[1] It is relatively abundant in soil bacterial colonies,[2–6] and its biological function is similar to that of glycogen in mammalian systems and starch in plants. PHB exists in the cytoplasmic fluid in the form of crystalline granules about 0.5 μm in diameter[2,7,8] and can be isolated as native granules[2,5,7] or by solvent extraction.[7,9] It was first identified about 60 years ago by the microbiologist Lemoigne, who made the early recognition that the reserve material was polymeric.[10] In some instances, however, the sudanophilic granules continued to be identified as a lipid storage material, since the concept of polymers was yet very novel. For example, in 1945 these granules were identified as fatty inclusions in *B. cereus*.[11] In 1958 in an infrared study of *B. megaterium* the following cautious statement was made: "some of the characteristics of the spectrum of vacuolated vegetative organisms are due to the presence of an extractable substance which may be a polymer of β-hydroxybutyric acid".[12] The infrared spectrum of the "lipid" sample in Figure 5b of this reference is very similar to the FTIR spectra obtained in the present study.

PHB is optically active, and molecular weights of a million or more have been reported.[1,13,14] As a thermoplastic it is suitable for biomedical applications or as a source of small-molecule specialty chemicals.[15,16] The high-purity material, lacking the catalyst residues typical of polyolefins from transition-metal-catalyzed polymerization, has been studied as a model system of spontaneous polymer nucleation and crystallization.[17,18] Because of its melting point (~179 °C), high molecular weight, and general characteristics similar to those of polyolefins,[13,14,19–22] it is being offered commercially as the "first thermoplastic from biotechnology".[23]

It is now apparent that PHB is actually just one of a family of microbial poly(β-hydroxyalkanoates),[3,24] all of which are potential sources of thermoplastic materials or small-molecule organic chemicals.[16,25] The Agricultural Division of Imperial Chemical Industries (ICI) has recently developed a controlled fermentation for the production of β-hydroxybutyrate and β-hydroxyvalerate copolyesters (1)



$$\left[\overset{*}{\underset{\displaystyle CH_3}{CH}}-CH_2-\overset{\displaystyle O}{\overset{\|}{C}}-O\right]_x\left[\overset{*}{\underset{\displaystyle CH_2CH_3}{CH}}-CH_2-\overset{\displaystyle O}{\overset{\|}{C}}-O\right]_y$$

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using a variety of feedstocks.[15,26] Acetic acid derived from these feedstocks is the building block for PHB homopolymer, whereas the addition of propionic acid to the growing culture results in poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) (P(HB-*co*-HV)).[9]

These copolyesters are available[9,23] in a range of compositions and are optically active. Since the physical properties vary systematically with composition,[9,27–29] it is important to have an accurate and precise method of composition analysis. Although the melting point varies with composition, this does not offer a reliable method since the copolyesters display a minimum in their melting point–composition curve.[30] In addition, the melting point shows a strong dependence on the thermal history of the materials. In our development of a method to determine the copolymer composition of P(HB-*co*-HV) samples by

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using Fourier transform infrared (FTIR) spectroscopy, it was discovered that this technique is also a sensitive probe of the degree of crystallinity of these copolyesters. Studies of the crystallization behavior of P(HB-*co*-HV) are especially interesting because these materials exhibit the unusual phenomenon of isodimorphism.[27,30] This behavior is typical of statistical copolymers where both monomers can crystallize and repeating units of one type are each included in the crystal lattice of the other. This paper describes the results of composition analysis by ^{1}H NMR and FTIR spectroscopy for a wide range of natural P(HB-*co*-HV) samples and discusses the crystallization behavior of these materials as investigated by FTIR spectroscopy and wide-angle X-ray diffraction.[31]

Experimental Section

Materials. The P(HB-*co*-HV) samples used in this study were produced by ICI, Agricultural Division, Billingham, UK, and are available under the trade name Biopol. Some of the materials are also available from Aldrich Chemical Co. The samples contained no added nucleating agents which would interfere with studies of crystallization kinetics. The as-received samples were fibrous powders, a result of the isolation process that involved methylene chloride or chloroform extraction of the polymer followed by precipitation into methanol.[7,9]

Methods. ^{1}H NMR spectra of 1–2 wt % solutions of P(HB-*co*-HV) in chloroform-*d* were recorded at 250 MHz on Bruker WM 250 and AM 250 spectrometers using DISNMR software. A 90° pulse angle and a relaxation delay of 2 s were employed over a spectral width of 12 ppm. Approximately 500 scans were acquired for copolyesters of low HV content, down to 32 scans for samples of high HV content. The FID was acquired at 16K and zero-filled to 32K before Fourier transformation. The copolymer composition, expressed as the mole percent β-hydroxyvalerate, is a ratio of peak areas due to the HV methyl resonance and the sum of the HB and HV methyl resonances. Compositions were determined by integration of the fully expanded spectra of the two methyl resonances of HB and HV units (for an example see part II of Figure 1).

FTIR spectra of the copolyesters were recorded with a Nicolet DX single-beam spectrometer interfaced with a Nicolet 20DX data processor. A total of 32 scans were recorded for most samples, but only 8 scans were acquired when time restrictions were imposed by kinetic studies. Samples were prepared by one of the following methods.

Solid copolyester films were cast from 1–2 wt % solutions in $CHCl_3$ on a KBr support. The point at which the solvent had visibly evaporated was taken as time zero for the purpose of kinetic studies. The samples were allowed to crystallize at room temperature.

KBr pellets using approximately 50 mg of IR spectroscopic grade KBr and 1 mg of as-received copolyester sample were prepared. The finely ground mixture was dried for 10 h in a vacuum oven at 60 °C. A Barnes QWIK Handi-Press was used to prepare thin KBr pellets 7 mm in diameter. IR absorption bands arising from surface moisture, still present even after extensive drying, were subtracted from the spectrum by an interactive background subtraction routine. As a result, shoulders on the C—H and C=O bands were removed, a procedure that was essential for accurate composition analysis.

Solutions of copolyesters were prepared in chloroform-*d* (1–2 wt %). The use of the deuteriated solvent avoided interference from solvent absorption in the aliphatic C—H stretch region around 2900 cm^{-1}. PHB homopolymer required heating to about 50 °C in order to dissolve completely. A solution cell with KBr windows and 0.1–0.2-mm spacers was used for all measurements. The FTIR spectrum of the solvent-filled cell served as the background, which was subtracted routinely from sample spectra.

FTIR composition analyses in the solid state of as-received samples containing 0–47 mol % HV were made by using the KBr pellet method. The C—H area was determined by integrating between 3130 and 2770 cm^{-1} and the C=O area by integrating between 2000 and 1580 cm^{-1}. The concentration of copolyester and the thickness of the pellet were varied in order to maintain the C=O absorbance between 0.7 and 1.1 absorbance units. As a result, the intensity of the C—H band was above 0.1 absorbance unit for all compositions. Outside the limits of 0.1–1.1 absorbance units (dynamic range), the relative error and hence the reproducibility became unacceptable.[32]

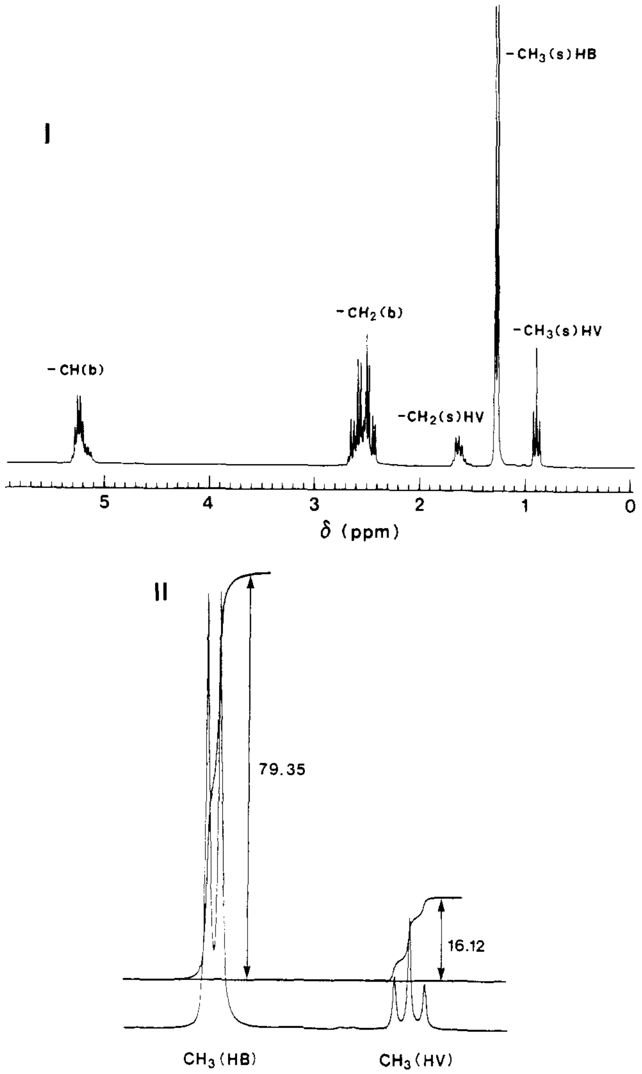

Figure 1. (I) 250-MHz ^{1}H NMR spectrum of a bacterial P(HB-*co*-HV) sample containing 17 mol % HV; b and s refer to backbone and side group, respectively; (II) expanded NMR spectrum of HB and HV methyl resonances of the same sample, with unnormalized integrated peak areas.

FTIR composition analyses in solution of samples containing from 0 to 47 mol % HV were performed in deuteriated chloroform. The C—H and C=O peak areas were determined by integrating between 3050 and 2800 and 1860 and 1640 cm^{-1}, respectively. Cell thickness and concentration were varied carefully in order to maintain spectra within the optimum intensity range of 0.1–1.1 absorbance units. For compositions ranging from 0 to 12 mol % HV it was found necessary for the C=O intensity to lie between 1.0 and 1.1 absorbance units, so that the C—H intensity would fall just within the optimum absorbance range. Above 12 mol % HV carbonyl intensities as low as 0.7 absorbance unit were acceptable.

Crystallization kinetics were followed by wide-angle X-ray diffraction (WAXS) on copolyesters ranging from 0 to 20 mol % HV. Samples were prepared by pressing the as-received powders in a diffraction holder up to 10 000 psi at approximately 5 °C above the melting point. The melting points of these materials ranged from 179 °C for PHB to 115 °C for P(HB-*co*-20% HV). Details of the thermal behavior of these copolyesters are reported elsewhere.[30] All samples were pressed and heated for 0.5 min and then immediately quenched in water at about 5 °C. This point was taken as time zero in the kinetic studies. Crystallization was

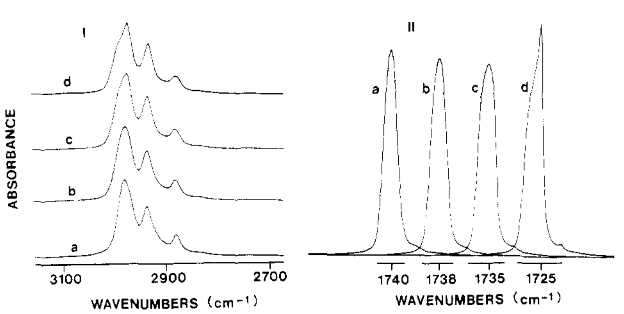

Figure 2. Effect of the degree of crystallinity on the peak area and shape of the (I) C—H and (II) C═O bands of a 20 mol % HV solution-cast copolyester (a) 1 min, (b) 40 min, (c) 65 min, and (d) 17 h after evaporation of the solvent. The C—H and the C═O bands were normalized to the band at 1382 cm^{-1}, which is insensitive to composition and degree of crystallinity.

allowed to proceed at room temperature. Crystallinity measurements were made by using a Philips Model PW 1050/80 X-ray powder diffractometer equipped with a graphite monochromator and pulse height analyzer. Nickel-filtered Cu Kα radiation (λ = 0.1542 nm) was used as the source. In addition, the crystallization kinetics of solution-cast films were followed by WAXS. Solutions of samples of P(HB-*co*-HV) in $CHCl_3$ were cast in a Teflon-coated container and allowed to dry for about 2 h. Time zero was approximated as the time at which the films were dry. Complete removal of $CHCl_3$ was confirmed by FTIR. The resulting films were mounted in a diffraction holder and allowed to crystallize at room temperature.

Results and Discussion

^{1}H NMR Composition Analysis. The 250-MHz ^{1}H NMR spectra for samples of PHB[33] and P(HB-*co*-HV) are well resolved, with spectral line widths on the order of 2 Hz. An example is given in part I of Figure 1. The compositions of 40 copolyester samples were determined from the areas of the methyl resonances in the side groups of HB and HV repeating units (see part II of Figure 1) and ranged from 0 to 47 mol % HV. Of these samples, 37 fell in the range of 0 to 21 mol % HV. The experimental uncertainty was estimated to be ±1 mol % HV. Repeated measurements on solutions of the same sample were routinely found to agree within ±0.5 mol % HV. In principle, one could also utilize the peak area of the methylene resonance due to the HV ethyl side group to determine copolymer compositions. However, this is not recommended since small amounts of water in either the polymer or the solvent lead to an impurity peak at ~1.67 ppm, which overlaps the methylene resonance of HV in $CDCl_3$. Hence, erroneously high copolymer compositions are obtained if water is present in the sample.

FTIR Composition Analysis. On the basis of the chemical structure of P(HB-*co*-HV) (1) one can predict that FTIR absorbances which will be sensitive to copolymer composition include the C—H bands and the C—C bands around 2900 and 977 cm^{-1}, respectively. The former are better resolved and hence preferred for composition analysis. In order to correlate spectra of different samples, the area of the C—H bands was normalized to the composition-insensitive C═O band. This normalized FTIR peak area can be directly related to the copolymer compositions previously determined by NMR. However, it is important to establish the conditions under which FTIR can be a precise and accurate technique for determining copolymer composition given that peak area, position, and shape are a function of degree of crystallinity. This is illustrated in parts I and II of Figure 2 for the C—H and C═O bands of a solution-cast copolyester film containing 20 mol % HV that was allowed to crystallize at room temperature. The most significant change in bandshape occurs between lines c and d, as a shoulder becomes apparent in both sections of the figure. Monitoring the sample by X-ray diffraction shows that structure on the C—H and C═O bands appears only when the sample has reached a significant level of crystallinity. This structure is the result of restricted orientation in the crystalline state. Integration results showed that the peak areas change significantly with the degree of crystallinity. Hence, composition analysis of these copolyesters must be carried out on samples having the same degree of crystallinity. This can be done in two ways: by carrying out the analysis on solid copolyesters that have all reached the same degree of crystallinity or by analyzing copolyesters in solution. Figure 3 shows the calibration curve for copolymer composition of as-received samples by FTIR in the solid state. These samples were shown by X-ray diffraction to be of similar degrees of crystallinity.[30] A correlation coefficient, r, of 0.994 was determined by linear regression analysis. The result of composition analysis of P(HB-*co*-HV) solutions in $CDCl_3$ is illustrated in Figure 4 (r = 0.998). The experimental uncertainty in copolyester composition determined by these FTIR methods was estimated to be ±2-3 mol % HV.

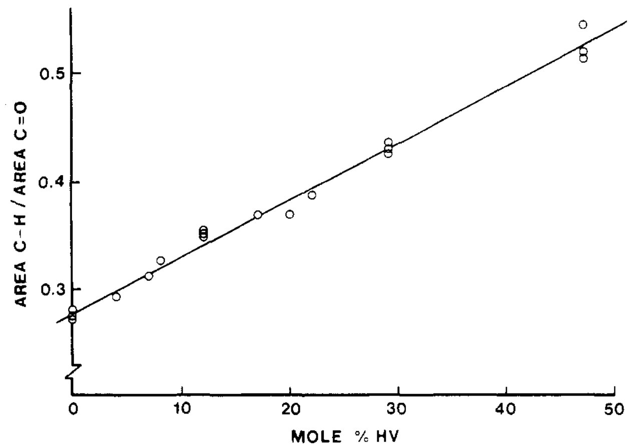

Figure 3. Variation of the ratio of the IR absorbance of the C—H band around 2900 cm^{-1} to the absorbance of the C═O band at 1735 cm^{-1} with composition for bacterial P(HB-*co*-HV) polyesters in the solid state. Compositions were determined by ^{1}H NMR spectroscopy. The IR spectra are recorded on as-received samples dispersed in KBr pellets.

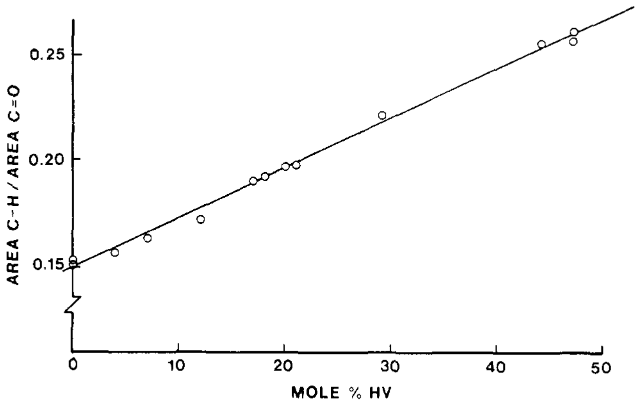

Figure 4. Variation of the ratio of the IR absorbance of the C—H band around 2900 cm^{-1} to the absorbance of the C═O band at 1735 cm^{-1} with composition of bacterial P(HB-*co*-HV) polyesters in chloroform-*d* solution. Compositions were determined by ^{1}H NMR spectroscopy.

The correlation of FTIR peak area of these two methods to composition determined by NMR measurements can be expressed as follows:

in the solid state, at a comparable degree of crystallinity

$$(\text{area C—H})/(\text{area C}{=}\text{O}) = ((5.27 \pm 0.14) \times 10^{-3})V + (0.279 \pm 0.004) \quad (1)$$

in chloroform-*d* solution

$$(\text{area C—H})/(\text{area C}{=}\text{O}) = ((2.37 \pm 0.05) \times 10^{-3})V + (0.150 \pm 0.001) \quad (2)$$

where V is the mol % HV of the P(HB-*co*-HV) sample.

A poor correlation ($r = 0.96$) was observed for solution-cast copolyester films that were analyzed 10 min after the solvent had evaporated. As shown below, these conditions lead to copolyester films of varying degrees of crystallinity depending on the HV content. It is concluded, therefore, that the calibration curves in Figures 3 and 4 are reliable as a result of comparable (or absence of) crystallinity in the samples used in each method.

Crystallinity Studies. Parts a and b of Figure 5 show part of the FTIR spectra of PHB homopolymer in chloroform-*d* solution and as a solution-cast film. A comparison of these spectra reveals significant differences between the two spectra which provide information on the crystallinity of the polyester. X-ray diffraction and polarizing microscopy on films such as those used to record the spectrum in Figure 5b clearly demonstrate that PHB is in a crystalline state immediately after solvent evaporation.[19] Particularly noteworthy are the differences in intensities of the bands at 1279, 1228, and 1185 cm^{-1} (marked with asterisks) between PHB in solution and in the solid state. In addition, the bands at 1134, 1102, and 1060 cm^{-1} are also sensitive to the degree of crystallinity, however, to a lesser extent. Parts c and d of Figure 5 show part of the FTIR spectra of a P(HB-*co*-20% HV) sample in solution and as a solution-cast film, respectively. The similarity of these two spectra indicates that the copolyester sample containing 20 mol % HV did not crystallize in the 10 min which elapsed between evaporation of the solvent and recording of the spectrum. However, wide-angle X-ray diffraction (WAXS) of as-received samples and aged solution-cast films of the copolyesters showed a high level of crystallinity (61–74 ± 5 %) at all compositions.[27,30] Consequently, FTIR spectra were recorded for a solid film of the 20% copolyester at different times after evaporation of the solvent. Representative spectra are shown in Figure 6. The peak intensities of the crystallinity-sensitive bands (indicated by arrows) change regularly with time as crystallite nucleation and growth occur. It is immediately evident that the 20% copolyester sample crystallizes much more slowly than PHB homopolymer when cast from solution. This observation led to a study of the crystallization kinetics of solution-cast copolyesters.

Changes in the degree of crystallinity were observed in the FTIR experiments by following the relative intensity of the band at 1185 cm^{-1}, which displays the largest difference in intensity between the crystalline and amorphous states and is better resolved than the other two crystallinity-sensitive bands at 1228 and 1279 cm^{-1}. This is illustrated in a more detailed comparison, shown in Figure 7, of part of the FTIR spectra of the 20% copolyester in amorphous and semicrystalline states. A relative measure of the degree of crystallinity was obtained by normalizing the absorbance at 1185 cm^{-1} to that of the 1382-cm^{-1} band, which is insensitive to the degree of crystallinity and copolymer composition. Hence, a crystallinity index, CI, was defined as the ratio of the intensities of the bands at 1382 and 1185 cm^{-1} (marked with asterisks in Figure 7). This crystallinity index is not to be confused with an absolute degree of crystallinity. The results of the kinetic study for solution-cast films are given in Figure 8. From this plot it can be seen that PHB homopolymer crystallizes rela-

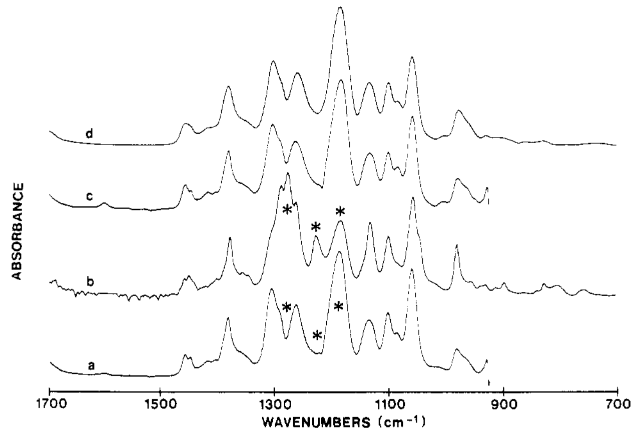

Figure 5. FTIR spectra of PHB homopolymer (a) in CDCl$_3$ solution and (b) as a solution-cast film 10 min after evaporation of the solvent; FTIR spectra of a P(HB-*co*-HV) sample containing 20 mol % HV (c) in CDCl$_3$ solution and (d) as a solution-cast film 10 min after evaporation of the solvent. The bands marked with asterisks are sensitive to the degree of crystallinity.

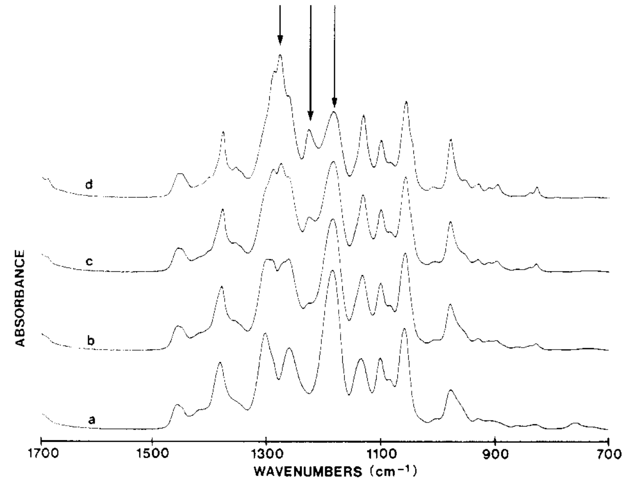

Figure 6. FTIR spectra of a solution-cast copolyester sample containing 20 mol % HV (a) 1 min, (b) 40 min, (c) 65 min, and (d) 17 h after evaporation of the solvent. The arrows indicate the bands that are most sensitive to the degree of crystallinity.

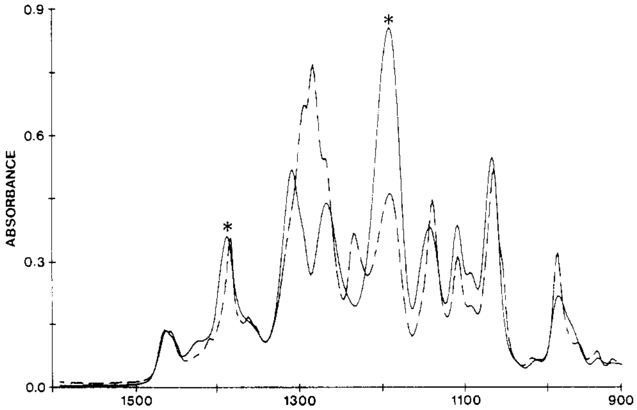

Figure 7. Comparison of crystallinity-sensitive bands of a solution-cast copolyester sample containing 20 mol % HV recorded 1 min (solid line) and 17 h (broken line) after evaporation of the solvent. The solid line corresponds to the amorphous sample and the broken line to the semicrystalline sample. Peaks marked with asterisks were used for calculating the crystallinity index, CI.

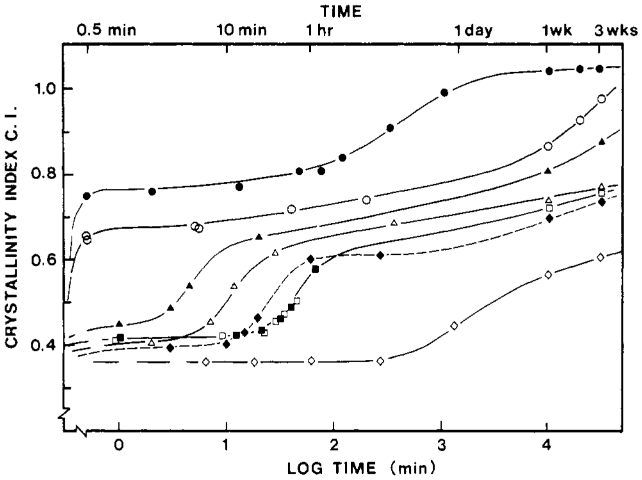

Figure 8. FTIR of samples of P(HB-*co*-HV) cast from $CHCl_3$ solution. Variation of crystallinity index, CI, with time and molar composition: 0% HV, ●; 7% HV, ○; 12% HV, ▲; 17% HV, △; 20% HV, ■; 21% HV, □; 29% HV, ◆; 47% HV, ◇.

Table I
Crystallinity Index (CI) of As-Received Bacterial P(HB-*co*-HV) Samples As Determined by FTIR in the Solid State[a]

mol % HV	CI[b]	mol % HV	CI[b]
0	0.99 (±0.08)	17	0.93 (±0.08)
4	0.97 (±0.08)	20	0.92 (±0.08)
7	0.98 (±0.08)	21	0.90 (±0.08)
8	0.96 (±0.08)	29	0.86 (±0.08)
12	0.97 (±0.08)	47	0.81 (±0.08)

[a] Spectra recorded by using KBr pellet method. [b] (Intensity at 1382 cm^{-1})/(intensity at 1185 cm^{-1}).

tively quickly; however, copolyesters of increasing HV content crystallize more and more slowly. The latter display an induction period in which the samples are virtually amorphous, whereas samples of low HV content start to crystallize within 1 min. The change in the rate of crystallization may reflect changes in nucleation rate or differences in the rate of crystal growth. The presence of an induction period, followed by significantly slower rates of crystallization for copolyesters with higher HV content, indicates that both processes are influenced by the copolymer composition. It is remarkable that the CI continues to increase even for copolyesters of low HV content long after initial crystallization has occurred; this probably reflects a process involving secondary crystallization that results in an increase in size or perfection of spherulites. The two-stage process suggests a first crop of highly ordered crystals followed by a second crop, probably much less perfect. Indeed calorimetric studies on copolyesters containing 4–21 mol % HV suggest that there are two populations of crystals separated in melting point by 10–30 °C. The results of annealing studies followed by differential scanning calorimetry (DSC) will be reported elsewhere.

Table I shows the crystallinity index measured by FTIR in the solid state for as-received copolyester samples. Values of CI do not change significantly for copolyester samples containing up to 47 mol % HV; these results confirm that the degree of crystallinity is virtually independent of HV content for solution-precipitated and aged P(HB-*co*-HV) samples, as X-ray studies have also shown.[27,30] It is concluded, therefore, that the solution-casting technique employed here imposes certain restrictions on the crystallization process since most values of CI, even after 3 weeks, are lower than those for as-received samples having the same HV content (cf. Figure 8; CI ranges from 0.60 for P(HB-*co*-47% HV) to 1.05 for PHB). To determine the rate of crystallization of these copolyesters when precipitated from solution, the equivalent of as-received materials were prepared from samples of PHB and P(HB-*co*-20% HV) by addition of a 1% solution in chloroform to the nonsolvent diethyl ether. Values of CI = 0.98 ± 0.08 were obtained by means of the KBr pellet technique for both samples directly following the precipitation. This observation indicates that precipitation of the copolyesters from solution, in contrast to casting of films from solution, results almost immediately in the optimum crystallinity.

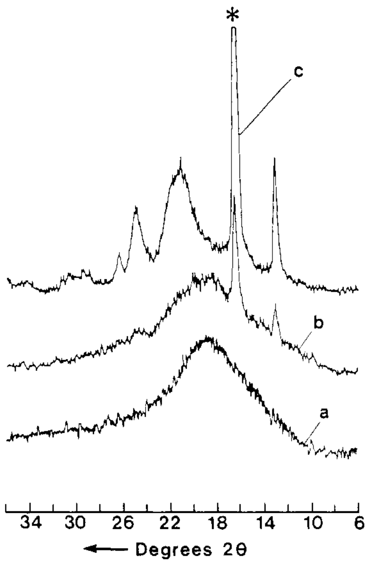

Figure 9. X-ray diffraction patterns of a melt-quenched copolyester sample containing 20 mol % HV after (a) 15 min, (b) 3 h, and (c) 1 week at room temperature.

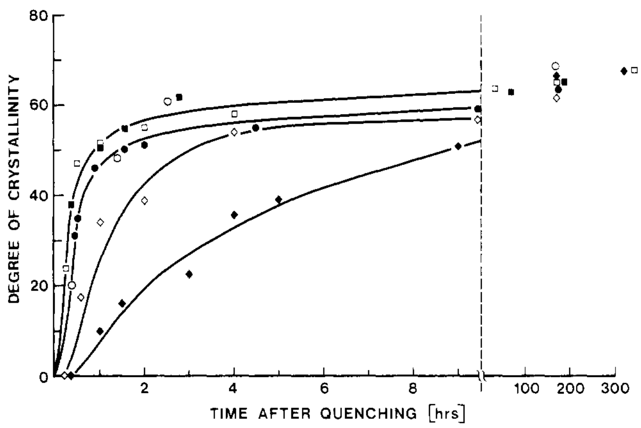

Figure 10. X-ray diffraction of melt-quenched samples of P-(HB-*co*-HV). Variation of the degree of crystallinity with time and molar composition: 0% HV, □; 4% HV, ■; 7% HV, ○; 8% HV, ●; 17% HV, ◇; 20% HV, ◆. From triplicate measurements on the diffraction traces the experimental uncertainty was estimated to be ±5%.

In addition to the IR study of crystallization kinetics of samples cast from solution, a similar study was carried out by wide-angle X-ray diffraction (WAXS) on copolyester samples quenched from the melt. Parts a and b of Figure 9 show that a melt-quenched copolyester sample containing 20 mol % HV is still amorphous after 15 min but that relatively weak crystalline bands are superimposed on the amorphous background scattering after 3 h. Eventually the crystalline peaks are well-defined in the X-ray diffractogram, as is seen in Figure 9c. The degree of crystallinity, X_c, was obtained from diffracted intensity data in the range $2\theta = 6$–36° by dividing the area of the crystalline peaks by the total area of the crystalline peaks and amorphous scattering. Figure 10 is a plot of the degree of crystallinity vs. time for copolyester samples ranging from 0 to 20 mol % HV (inadequate amounts of the 29 and 47 mol % HV samples prohibited their incorporation in this study). Clearly, the same trend of decreasing rate of crystallization with increasing HV content exists for samples of P(HB-*co*-HV) quenched from the melt as was observed for solution-cast copolyesters. Also, these data reemphasize that the equilibrium values of X_c, ranging from 62 to 69%, correspond closely for all compositions. Hence, the equilibrium X-ray crystallinity is approximately the same for all P(HB-*co*-HV) samples, as is also shown by independent measurements in the companion paper.[30]

According to the Scherrer equation the crystallite size is inversely proportional to the width at half-height, β, of, for example, the crystalline peaks at $2\theta \simeq 17°$ (marked with an asterisk in Figure 9). It was found that β remained virtually unchanged for all melt-quenched samples. This indicates that crystallization from the melt is predominantly nucleation rate limited and emphasizes the high purity of these bacterial thermoplastics, as noted previously for PHB homopolymer by Barham et al.[17,18] As a verification of the FTIR studies, the crystallization of solution-cast samples of P(HB-*co*-HV) containing 4 and 20 mol % HV was also followed by WAXS. It was found that the initial value of β was about twice that of the melt-quenched samples but decreased to this value in the so-called secondary crystallization phase. These results imply that crystallization of these copolyesters is mainly nucleation rate limited in the first phase and limited by the rate of crystal growth in the second phase. Optical microscopy of P(HB-*co*-HV) films cast from solution, between crossed polarizers, has shown that the spherulite size increases with increasing HV content.[31] This is because the nucleation rate decreases with increasing HV content.

Conclusion

The convenience of Fourier transform IR spectroscopy (FTIR) as an analytical technique for copolyester composition analysis is demonstrated in this study. While analysis using 250-MHz ^{1}H NMR is more accurate, the FTIR methods make use of an instrument more likely to be on hand in an industrial laboratory. However, the influence of crystallinity on IR band intensities requires that the samples be in solution if reliable results are to be obtained. The fact that comparable results could be obtained for the as-received samples of P(HB-*co*-HV) by using the KBr disk method is a consequence of the nearly equal level of crystallinity in all of these samples. These as-received copolyesters result from an isolation process involving solution precipitation, in which all of these samples attained about the same degree of crystallinity. The fact that copolymers containing almost 50 mol % HV are highly crystalline is a result of their remarkable isodimorphic behavior, a subject which is covered in more detail in another paper.[30]

A comparison of FTIR spectra of P(HB-*co*-HV) samples in solution and in the solid state allowed identification of bands sensitive to the degree of crystallinity. The most important spectral differences between amorphous and semicrystalline samples include intensity changes of bands at 1279, 1228, and 1185 cm^{-1}. The band at 1185 cm^{-1} was used to monitor crystallization kinetics of solution-cast films of P(HB-*co*-HV), and it was shown that the rate of crystallization of these copolyesters decreased with increasing HV content. This behavior agreed with wide-angle X-ray diffraction (WAXS) studies, in which the same trend was observed for copolyester samples quenched from the melt. Although crystallization of these copolyesters from the melt is faster than for solution-cast samples, both processes are slow when compared to the rate of crystallization of solution-precipitated samples. Moreover, these processes are slow in comparison with semicrystalline polyolefins. There have been reports that the degree of crystallinity, as measured by WAXS, decreases with HV content for copolyesters cast from solution.[28,29] Our results suggest instead that this apparent decrease in degree of crystallinity with increasing HV content arises because solution-cast films of higher HV content take much longer to reach the same high degree of crystallinity. For practical applications of these bacterial thermoplastics the crystallization rate can be greatly increased by the addition of nucleating agents, a procedure that also reduces brittleness and increases elongation at break.

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Isodimorphism in Bacterial Poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate)

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ABSTRACT: Bacterial copolymers from *Alcaligenes eutrophus* containing β-hydroxybutyrate and β-hydroxyvalerate monomer units were characterized by X-ray diffraction, differential scanning calorimetry, solution NMR, and solid-state ^{13}C NMR. The copolyesters were shown to be statistically random and of high crystallinity (>60%) throughout a range of compositions varying from 0 to 47 mol % β-hydroxyvalerate units. A minimum in the melting point vs. composition curve was found at approximately 30 mol % β-hydroxyvalerate. Only two crystal forms were detected: the β-hydroxybutyrate crystalline phase on one side of the melting point minimum and the β-hydroxyvalerate crystalline phase on the other. Solid-state ^{13}C NMR spectra support the conclusion that the poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) system is isodimorphic with each crystalline phase accommodating the repeating unit of the other monomer as part of its organized structure.

Introduction

This study involves the characterization of optically active copolyesters (Figure 1) of β-hydroxybutyrate (HB) and β-hydroxyvalerate (HV) as well as the reference homopolyester, poly(β-hydroxybutyrate) (PHB). These materials have recently been introduced by Imperial Chemical Industries (ICI) as biotechnology products with thermoplastic properties.[1] This is the first time that PHB has been available in commercial quantities.

Recently some of us reported[2] on the physical properties of chiral PHB and poly(β-hydroxyvalerate) (PHV) derived from sewage sludge.[3] In that case, it was concluded that the isolated polymeric material was a physical mixture of the two homopolymers. In the present study, X-ray diffraction, differential scanning calorimetry, solution NMR, and solid-state ^{13}C NMR were used to characterize a series of P(HB-*co*-HV) samples, which had been obtained from homogeneous bacterial cultures by a patented procedure.[4] These copolyesters have a statistically random distribution of comonomer units and approximately the same high degree of crystallinity, while exhibiting a minimum in their melting point vs. composition curve. The phenomenon explaining this behavior is called isodimorphism.[5] In this paper data are presented to substantiate the hypothesis of P(HB-*co*-HV) isodimorphism.

Experimental Section

Materials. The PHB and P(HB-*co*-HV) samples used in this work were obtained from Dr. P. A. Holmes, ICI Agricultural Division, Billingham, UK. Similar materials are commercially available from ICI under the trade name Biopol. As received, the samples were semicrystalline powders that had been isolated from *Alcaligenes eutrophus* cultures and precipitated from chloroform solution by the addition of methanol.[4] The range of P(HB-*co*-HV) compositions, as measured by ^{1}H NMR spectroscopy,[6] was 0–47 mol % HV.

NMR Spectroscopy. Solution ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer operating at 250.13 MHz for ^{1}H and 62.9 MHz for ^{13}C. Samples were prepared in chloroform-*d* (10–20 mg/mL for ^{1}H, 40–50 mg/mL for ^{13}C) containing tetramethylsilane (Me_4Si, $\delta = 0$) as an internal chemical shift standard. Proton spectra were recorded at ambient temperature (23 °C) with a spectrum width of 2500 Hz, 32K data points, a 45° pulse (2 μs), and 16–128 transients. Proton-decoupled ^{13}C spectra were obtained at 32 °C with a 12500-Hz spectral width, 32K data points, a 45° pulse (12.5 μs), and typically 15000–30000 transients. The ^{13}C FID was zero-filled three times before Fourier transformation to provide adequate digital resolution (0.095 Hz/point) to accurately define the peak line shape. Resolution-enhanced spectra were obtained by a Lorentz–Gauss transformation of the FID[7] using a line broadening of −1 to −1.5 Hz and a Gaussian multiplication factor of 0.3–0.4. Peak areas were determined from unenhanced spectra by spectrometer integration and/or by curve fitting. The latter procedure utilized LINESIM,[8] an interactive Pascal program for the Bruker Aspect 2000 computer. A sum of Lorentzian lines of equal but variable line width was visually fit to the experimental bandshape by using the residual sum of squares as a goodness-of-fit criterion. Individual peak areas were calculated from the height and width values for the optimized fit.

Solid-state ^{13}C cross-polarization magic-angle-spinning (CP/MAS) NMR spectra were recorded at 22.6 MHz on a Bruker CXP-100 spectrometer using a home-built probe and room-temperature spinning apparatus. Measurements were made for as-received powder samples and films cast from chloroform solution. Stretched films were prepared by extension to break and annealed samples by heating at 20 °C below the melting point for 1 h after stretching. CP/MAS spectra were obtained with spin locking and decoupling fields of ~10 G and spinning rates of ~2.5 kHz. A 1-ms contact time and 1-s recycle delay were used, yielding semiquantitative results.[9] Chemical shifts were referenced to external hexamethyldisiloxane (by substitution) and converted to the Me_4Si scale by adding 2.1 ppm to the measured values.

Thermal Analysis. Thermal data were recorded on a Perkin-Elmer DSC-2C instrument previously calibrated with an indium standard. Samples were annealed by heating at 20 °C/min to within 20 °C of the melting point and then cooling at 20 °C/min to room temperature. DSC traces were recorded at 20, 10, and 5 °C/min; slower heating rates were not used to avoid thermal degradation of the polyester sample. Melting points were determined by extrapolation to zero heating rate. Where multiple endotherms were observed, the melting point from the higher temperature endotherm was taken as the true melting point.

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